Journal of Enzyme Inhibition and Medicinal Chemistry, 2003 Vol. 18 (1), pp. 41–45

Taylor & Francis
healthsciences

Evaluation of 5-[1-(2-Halo(or nitro)ethoxy-2-iodoethyl)]-2′-deoxyuridines as Inhibitors of Herpes Simplex Virus

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(Received 25 June 2002)

A series of 5-[1-(2-haloethyl(or nitro)ethoxy-2-iodoethyl)]-2′-deoxyuridines (3–7) and related uracil analogs (9–10) were prepared using 5-vinyl-2′-deoxyuridine (2) and 5-vinyl uracil (8) as starting materials. The regiospecific reaction of 2 and 8 with iodine monochloride and an alcohol provided the target compounds 3–10. These analogs were evaluated in vitro for inhibitory activity against thymidine-kinase (TK) positive and negative strains of herpes simplex virus type-1. The compounds 3–10 were either weak or non-inhibitory to HSV-1 replication. All compounds investigated exhibited low host cell cytotoxicity.

Keywords: Inhibitors; Antiviral activity; Pyrimidine nucleosides; 5-Substituted-2′-deoxyuridines; 5-Substituted uracils; Drug design

INTRODUCTION

Herpes viruses' infections are serious and often life threatening to immuno-compromised individuals including solid organ or bone marrow transplant recipients and persons with acquired immuno deficiency syndrome (AIDS) whose numbers are increasing significantly. Herpes simples virus type 1 (HSV-1) and type 2 (HSV-2) infections lead to orolabial, genital and anorectal mucocutaneous disease, esophagitis and life-threatening encephalitis [central nervous system (CNS) infection].[1] At the present time, these viruses are not controlled by vaccination. The search to find new approaches for the treatment and suppression of herpes viruses in patients with AIDS has to be continued for the management of these infections.

Acyclovir is a widely used drug for the treatment of opportunistic herpes virus infections in immunocompromised patients. However, resistance to acyclovir is increasing, due to altered or deficient thymidine kinase enzymes in the resistant viruses.[2,3] Herpes virus thymidine kinase (HSV-TK) leads to initial selective phosphorylation of acyclovir resulting in a monophosphate derivative of the drug. However, the herpes virus infected cells still contain host encoded TK, and nucleoside analogs, which work as anti-herpes agents independent of HSV-TK, can be identified and potentially used for acyclovir resistant HSV infections. It has been reported that a number of acyclovir resistant clinical isolates of HSV-1 do not phosphorylate acyclovir, but they can phosphorylate thymidine and other pyrimidine nucleoside analogs.[4]

In our earlier studies,[5] it was reported that 5-(1-methoxy-2-iodoethyl) derivative of 2′-deoxyuridine (**1a**) exhibited potent anti-HSV-1 activity. This compound also possessed remarkable activity against Epstein Barr virus (EBV), which was 10-time superior to acyclovir.[5] It has been suggested that EBV encodes for very low levels of viral thymidine kinase activity. In our recent studies,[6] 5-(1-cyanamido-2-iodoethyl)-2′-deoxyuridine (**1b**) exhibited marked activity against both TK^- and TK^+ strains of HSV-1, and had low host cell toxicity. The exact mechanism of action of this compound is not yet known. However, it can be postulated that it may be phosphorylated by cellular kinases followed by selectively inhibition of viral DNA polymerase. These results indicate that novel substituents at C-1 of the 5-side chain of 2′-deoxyuridine may confer anti-herpes activity, independent of the thymidine kinase. In order to obtain further information on structural requirements at the C-1 position, we have

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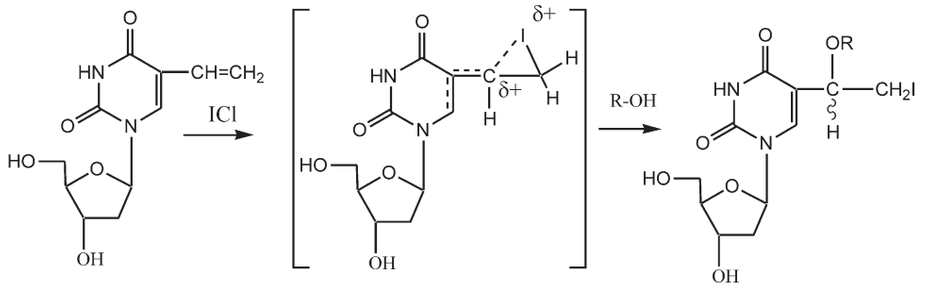
ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.1080/1475636031000062865

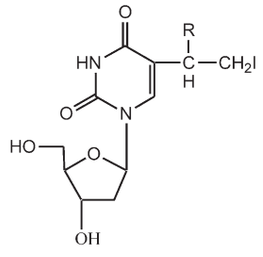


2

3, R = CH_2CH_2Br
4, R = CH_2CH_2Cl
5, R = $CH_2CH_2NO_2$
6, R = CH_2CBr_3
7, R = CH_2CCl_3

SCHEME 1

designed and synthesized a new series of 5-(1-alkoxy-2-iodoethyl) derivatives of 2′-deoxyuridine (**3**–**7**) and uracil (**9**,**10**) as potential inhibitors of HSV-1.



1a, R = OCH_3
1b, R = NHCN

MATERIALS AND METHODS

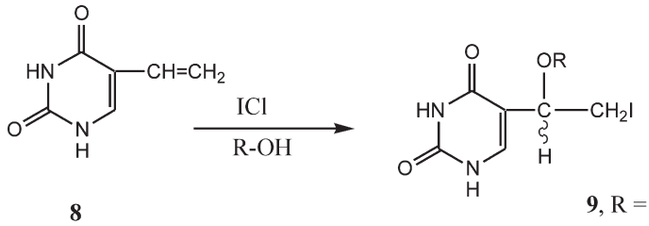
Chemistry

The target 5-[1-(2-haloethyl(or nitro)ethoxy-2-iodoethyl)] (**3**–**7**) derivatives of 2′-deoxyuridine were synthesized, by the regiospecific reaction of the 5-vinyl-2′-deoxyuridine (**2**) with iodine monochloride and an alcohol such as 2-bromo(or chloro, nitro) ethanol, 2,2,2-tribromoethanol, or 2,2,2-trichloroethanol in 24–52% yields as illustrated in Scheme 1. The products **3**–**7** exist as mixture of two diastereomers in a ratio of 1:1, which differ in configuration (*R* or *S*) at the 1 position of the 5-(1-alkoxy-2-iodoethyl) substituent that could not be separated by thin layer or column chromatography. The regiospecific addition is consistent with the results of Dalton *et al.*,[7] in which unsymmetrical olefins, capable of halonium ion formation were found to favor an unsymmetrical bridged intermediate of the type illustrated in Scheme 1, even in solvents having a high dipole moment.

Similar reactions of 5-vinyl uracil (**8**) with iodine monochloride in the presence of 2-bromoethanol or 2,2,2-tribromoethanol afforded the respective 5-[1-(2-bromoethoxy)-2-iodoethyl] (**9**) and 5-[1-(2,2,2-tribromoethoxy)-2-iodoethyl] (**10**) derivatives of uracil in 22–47% yields as illustrated in Scheme 2.

Experimental

Nuclear magnetic resonance spectra (^{1}HNMR) were determined on a Bruker AM-300 spectrometer using Me_4Si as an internal standard. Silica gel column chromatography was carried out using Merck 7734 silica gel (100–200 micro particle size). Thin layer chromatography (TLC) was performed with



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9, R = CH_2CH_2Br
10, R = CH_2CBr_3

SCHEME 2

Whatman MK6F silica gel microslides. 5-Vinyl-2′-deoxyuridine (**2**)[6] and 5-vinyl uracil (**8**)[8] were prepared using the literature procedures.

5-[1-(2-Bromoethoxy)-2-iodoethyl]-2′-deoxyuridine (3)

A solution of ICl (81 mg, 0.5 mmol) in 2-bromoethanol (1 ml) was added to a solution of **2** (125 mg, 0.49 mmol) in 2-bromoethanol (3 ml) and the reaction was allowed to proceed at 50°C for 45 min with stirring. Removal of the solvent *in vacuo* and elution of the product from a silica gel column using chloroform–methanol (97:3, v/v) as eluent yielded **3** (75 mg, 30%) as a syrup. ^{1}H NMR (D_2O) (mixture of two diastereomers in a ratio of 1:1) δ 2.18–2.32 (m, 2H, H-2′), 3.42–3.55 (m, 2H, CH_2I), 3.57–3.75 (m, 6H, H-5′, OCH_2CH_2Br), 3.86–3.96 (m, 1H, H-4′), 4.24–4.38 (m, 2H, H-3′, $CHCH_2I$), 6.10 and 6.16 (two t, J = 6 Hz, 1H total, H-1′), 7.72 and 7.74 (two s, 1H total, H-6). Anal. Calcd. for $C_{13}H_{18}BrIN_2O_6$: Found: C, 30.65; H, 3.45; N, 5.24; requires: C, 30.91; H, 3.59; N, 5.54%.

5-[1-(2-Chloroethoxy)-2-iodoethyl]-2′-deoxyuridine (4)

A solution of ICl (96 mg, 0.6 mmol) in 2-chloroethanol (1 ml) was added to a solution of **2** (101 mg, 0.4 mmol) in 2-chloroethanol (3 ml) and the reaction was allowed to proceed at 50°C for 45 min with stirring. Removal of the solvent *in vacuo* and elution of the product from a silica gel column using chloroform–methanol (92:8, v/v) as eluent yielded **4** (100 mg, 52%) as a syrup. ^{1}H NMR (D_2O) (mixture of two diastereomers in a ratio of 1:1) δ 2.2–2.40 (m, 2H, H-2′), 3.2–3.34 (m, 2H, CH_2I), 3.42–3.57 (m, 2H, H-5′), 3.60–3.80 (m, 4H, OCH_2CH_2Cl), 3.90–4.0 (m, 1H, H-4′), 4.24–4.40 (m, 2H, H-3′, $CHCH_2I$), 6.18 and 6.22 (two t, J = 6 Hz, 1H total, H-1′), 7.72 and 7.80 (two s, 1H total, H-6). Anal. Calcd. for $C_{13}H_{18}ClIN_2O_6$: Found: C, 33.75; H, 3.90; N, 6.28; requires: C, 33.89; H, 3.93; N, 6.08%.

5-[1-(2-Nitroethoxy)-2-iodoethyl]-2′-deoxyuridine (5)

A solution of ICl (96 mg, 0.6 mmol) in dry acetonitrile (2 ml) was added slowly to a solution of **2** (150 mg, 0.59 mmol) in acetonitrile (15 ml) and 2-nitroethanol (3 ml) and the reaction was allowed to proceed at 45°C for 15 min with stirring. Removal of the solvent *in vacuo* and elution of the product from a silica gel column using chloroform–methanol (97:3, v/v) as eluent yielded **5** (80 mg, 29%) as a syrup. ^{1}H NMR (D_2O) (mixture of two diastereomers in a ratio of 1:1) δ 2.20–2.40 (m, 2H, H-2′), 3.2–3.36 (m, 2H, CH_2I), 3.42–3.78 (complex m, 6H, H-5′, $OCH_2CH_2NO_2$), 3.90–4.03 (m, 1H, H-4′), 4.30–4.40 (m, 1H, H-3′), 4.58–4.66 (m, 1H, $CHCH_2I$), 6.16 and 6.20 (two t, J = 6 Hz, 1H total, H-1′), 8.74 and 8.77 (two s, 1H total, H-6). Anal. Calcd. for $C_{13}H_{18}IN_3O_8$: Found: C, 33.0; H, 3.80; N, 8.90; requires: C, 33.13; H, 3.84; N, 8.91%.

5-[1-(2,2,2-Tribromoethoxy)-2-iodoethyl]-2′-deoxyuridine (6)

A solution of **2** (40 mg, 0.16 mmol) in acetonitrile (5 ml) and 2,2,2-tribromoethanol (113 mg, 0.4 mmol) was stirred at 25°C for 15 min. A solution of ICl (32.4 mg, 0.2 mmol) in acetonitrile (3 ml) was added to the clear solution previously prepared and the reaction was allowed to proceed at 35°C for 15 min with stirring. Removal of the solvent *in vacuo* and elution of the product from a silica gel column using chloroform–methanol (95:5, v/v) as eluent yielded **6** (25 mg, 24%) as a syrup. ^{1}H NMR (CD_3OD) (mixture of two diastereomers in a ratio of 1:1) δ 2.30–2.46 (m, 2H, H-2′), 3.14–3.23 (m, 1H, $CHH'I$), 3.32–3.44 (m, 2H, H-5′), 3.52–3.60 (m, 1H, $CHH'I$), 3.88–3.95 (m, 1H, H-4′), 4.14 (s, 2H, OCH_2CBr_3), 4.58–4.68 (m, 2H, H-3′, $CHCHH'I$), 6.28 and 6.32 (two t, J = 6 Hz, 1H total, H-1′), 8.01 and 8.03 (two s, 1H total, H-6). Anal. Calcd. for $C_{13}H_{16}Br_3IN_2O_6$: Found: C, 23.45; H, 2.23; N, 4.02; requires: C, 23.55; H, 2.43; N, 4.22%.

5-[1-(2,2,2-Trichloroethoxy)-2-iodoethyl]-2′-deoxyuridine (7)

A solution of **2** (150 mg, 0.59 mmol) in acetonitrile (5 ml) and 2,2,2-trichloroethanol (6 ml) was stirred at 25°C for 15 min. A solution of ICl (96 mg, 0.6 mmol) in acetonitrile (1 ml) was added to the clear solution previously prepared and the reaction was allowed to proceed at 50°C for 5 min with stirring. Removal of the solvent *in vacuo* and purification of the product by elution from a silica gel column using dichloromethane–methanol (98:2, v/v) as eluent yielded **7** (80 mg, 28%) as a syrup. ^{1}H NMR (Me_2SO-D_6) (mixture of two diastereomers in a ratio of 1:1) δ 2.04–2.22 (m, 2H, H-2′), 3.42–3.70 (m, 4H, H-5′, $CHCH_2I$), 3.73–3.84 (m, 1H, H-4′), 4.20–4.30 (m, 2H, OCH_2CCl_3), 4.64–4.74 (m, 2H, H-3′, $CHCH_2I$), 6.20 and 6.22 (two t, J = 6 Hz, 1H total, H-1′), 7.86 and 7.88 (two s, 1H total, H-6), 11.50 (s, 1H, NH, exchanges with deuterium oxide). Anal. Calcd. for $C_{13}H_{16}Cl_3IN_2O_6$: Found: C, 29.25; H, 3.0; N, 5.35; requires: C, 29.47; H, 3.04; N, 5.29%.

5-[1-(2-Bromoethoxy)-2-iodoethyl]-uracil (9)

A solution of ICl (96 mg, 0.6 mmol) in acetonitrile (1 ml) was added to a solution of **8** (80 mg, 0.58 mmol) in acetonitrile (5 ml) and 2-bromoethanol (1 ml). The reaction was allowed to proceed at 50°C

for 15 min, the solvent was removed *in vacuo*, and the product was purified by elution from a silica gel column using dichloromethane–methanol (98:2, v/v) as eluent to afford **9** (50 mg, 22%) as a syrup. ^{1}H NMR (Me_2SO-D_6) δ 3.35–3.42 (m, 1H, C*H* H′I), 3.45–3.52 (m, 1H, CH*H*′I), 3.55–3.65 (m, 2H, OCH_2CH_2Br), 3.70–3.80 (m, 2H, OCH_2CH_2Br), 4.46–4.52 (m, 1H, C*H*CHH′I), 7.40 (d, $J_{NH,6} = 6.2$ Hz, 1H, H-6, collapses to a singlet after exchange with deuterium oxide), 11.4 (d, $J_{NH,6} = 6.2$ Hz, 1H, N^1-H, exchanges with deuterium oxide), 11.24 (s, 1H, N^3-H, exchanges with deuterium oxide). Anal. Calcd. for $C_8H_{10}BrIN_2O_3$: Found: C, 24.33; H, 2.50; N, 7.25; requires: C, 24.70; H, 2.59; N, 7.20%.

5-[1-(2,2,2-Tribromoethoxy)-2-iodoethyl]-uracil (10)

A solution of ICl (80.8 mg, 0.5 mmol) in dry acetonitrile (1 ml) was added to a solution of **8** (65 mg, 0.47 mmol) in acetonitrile (3 ml) and 2,2,2-tribromoethanol (283 mg, 1.0 mmol). The reaction was allowed to proceed at 50°C for 10 min with stirring, the solvent was removed *in vacuo*, and the product was purified by elution from a silica gel column using dichloromethane–methanol (98:2, v/v) as eluent to afford **10** (54.6 mg, 47%) as a syrup. ^{1}H NMR ($CDCl_3$) δ 3.38–3.50 (m, 1H, C*H* H′I), 3.50–3.65 (m, 1H, CH*H*′I), 4.28 (br s, 2H, OCH_2CBr_3), 4.60–4.63 (m, 1H, C*H*CHH′I), 7.45 (s, 1H, H-6). Anal. Calcd. for $C_8H_8Br_3IN_2O_3$: Found: C, 17.51; H, 1.50; N, 5.0; requires: C, 17.57; H, 1.47; N, 5.12%.

In Vitro Antiviral Assays [HSV-1 (KOS, TK^+), (KOSSB, TK^-)]

African green monkey kidney (Vero) cells were grown in DMEM supplemented with 5% FBS. Cells were seeded into 24-well plates one day prior to the assay. Wild-type HSV-1, KOS, and a thymidine kinase deficient mutant, KOSSB,[9] were used to infect at ~100 PFU's per well for 1 h at 37°C. After infection, the inoculum was replaced with serial dilutions of media containing diluted drug. All drug dilutions were done in duplicate, initially using the following concentrations: 50, 25, 10, 5 and 1 μg/ml (for TK^+ virus) and 50, 25, 10 and 1 μg/ml (for TK^- virus). Once an activity range was determined, compounds were again tested at 1:2 serial dilutions to obtain a more precise EC_{50}. Controls included infected wells that were not treated with drugs, as well as infected wells treated with acyclovir at 5, 1 and 0.1 μg/ml (for TK^+ virus) and 50, 25, 10 and 1 μg/ml (for TK^- virus). Plates were incubated for 48 h at 37°C. To visualize plaques, wells were fixed by incubation with methanol for 10 min at room temperature. The methanol was aspirated and replaced with 1× Giemsa stain (Sigma) for 1 h at room temperature. Plaques were counted and compared to the number of plaques in the no-drug controls in order to calculate EC_{50}.

Cell Cytotoxicity

MTT Assay

Cell viability was measured using the cell proliferation kit 1 (MTT; Boehringer Mannheim), as per manufacturer's instructions. Briefly, a 96 well plate was seeded with Vero cells at a density of 2.5×10^4 cells per well. Cells were allowed to attach for 6–8 h, the media was replaced with media containing drugs at concentrations of 100, 50, 25, 12.5, 6.3 and 1.5 μg/ml. Dimethyl sulfoxide (DMSO) was also included as control. Plates were incubated for 3 days at 37°C. The color reaction involved adding 10 μl MTT reagent per well, incubating 4 h at 37°C and then adding 100 μl solubilization reagent. Plates were read on an ELISA plate reader (Abs 560–650 nm) following an overnight incubation at 37°C.

RESULTS AND DISCUSSION

The antiviral activity for the new series of 5-[1-(2-halo(or nitro)ethoxy-2-iodoethyl)]-2′-deoxyuridines (**3**–**7**) and related uracils (**9**,**10**) were determined in culture against TK-positive HSV-1 (strain KOS) and mutant TK-deficient HSV-1 (strain KOSSB), by measuring their ability to inhibit the virus-induced cytopathic effect in Vero cells infected with HSV-1. The 5-[1-(2-bromoethoxy)-2-iodoethyl)] (**3**), 5-[1-(2-chloroethoxy)-2-iodoethyl)] (**4**), 5-[1-(2-nitroethoxy)-2-iodoethyl)] (**5**), and 5-[1-(2,2,2-tribromoethoxy)-2-iodoethyl)] (**6**) exhibited EC_{50} values of 20, 25, 5–10 and 25 μg/ml, respectively, against TK^+ strain of HSV-1, relative to the reference drug acyclovir ($EC_{50} = 0.2$ μg/ml). In contrast, compounds **3**–**7** were not inhibitory for TK-deficient strain of HSV-1 except compound **5** that exhibited moderate inhibitory effect with an EC_{50} of 10–25 μg/ml. These test results indicate that increasing the carbon chain at C-1 position of the 5-substituent of 2′-deoxyuridine is detrimental to anti-herpes activity since 5-(1-methoxy-2-iodoethyl)-2′-deoxyuridine (**1a**)[5] exhibited anti-HSV-1 activity approaching the potency of acyclovir. The uracil analogs **9** and **10** were found to be non-inhibitory against both TK^+ and TK^- strains of HSV-1, up to concentrations of 25 and 50 μg/ml, respectively. No cytotoxicity of the compounds (**3**–**10**) was observed towards uninfected Vero cells up to >100 μg/ml.

The weak anti-HSV-1 activity displayed by compounds **3**–**7** could be attributed to insufficient phosphorylation of these compounds by host and/or viral-encoded thymidine kinase, and/or lack of interaction with herpes encoded DNA polymerase.

The structure activity relationship studies indicate that larger substituents at C-1 of the 5-side chain of 2′-deoxyuridine may not be well tolerated by the herpes virus enzymes, resulting in low antiviral activity.

Acknowledgements

We are grateful to the Alberta Heritage Foundation for Medical Research (AHFMR, Canada) for an establishment grant and the Canadian Institutes of Health Research (CIHR) for operating grants (R.K.). R.K. is a recipient of an AHFMR Medical Scholar Award.

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